REMARKS

Applicants again thank the Examiner for the courtesy of a telephonic interview with Applicants' undersigned attorney on December 21, 2004. It was proposed at that time to positively recite strain-controlled deformational loading in all independent claims to distinguish over the prior art, to which the Examiner seemed receptive.

In a previously filed Amendment responsive to the final Office Action dated September 21, 2004, Applicants amended the claims herein in a manner believed consistent with what the Examiner had indicated was acceptable. However, in an Advisory Action dated April 28, 2005 entry of said Amendment was refused due to new issues, etc. While Applicants respectfully disagree regarding whether there were any new issues, Applicants believe the present Amendment reflects additional changes made to the claims in response to the Examiner's comments in the Advisory Action.

In the amendments above, Claims 1, 6, 7, 11, 29, 30, 36, 38-40, 43, 44, and 61 have been amended, and new Claims 62 and 63 have been added, to more particularly point out and distinctly claim Applicants' invention.

In the Office Action dated September 21, 2004, Claims 29 and 30 were objected to. The Examiner's attention is directed to the amendments above, wherein Claims 29 and 30 have been amended to overcome the objection.

Claims 11, 28, 29, and 31 to 60 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Examiner's attention is again directed to the amendments above, wherein the bases of the §112 rejection are believed to have been overcome.

Claim 30 has been objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim should refer to other claims in the alternative only. The Examiner's attention is directed to the amendments above, wherein Claim 30 has been amended.

Claims 1-29 and 31-61 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner maintains that Claims 1 and 61 recite that the device include "means for applying hydrostatic fluid pressure and/or strain-controlled deformational loading via loading platens"; that this language is indefinite because it cannot be clearly determined if the recited loading platens apply both the recited hydrostatic fluid pressure and strain-controlled deformational loading or just the strain-controlled loading; that review of the instant specification would indicate that the platens merely apply the strain-controlled loading; that, as a result, the claim could be interpreted as a device that only includes a "means for applying hydrostatic loading" in view of the use of the language "and/or" in the claim.

The Examiner's attention is directed to the amendments above, wherein the amendments to Claims 1 and 29 are believed to overcome this rejection.

Claims 1-6, and 22-27 have been rejected under 35 U.S.C. §102(e) as being anticipated by Peterson et al., U.S. Patent No. 6,121,042 ("Peterson"). The Examiner maintains that with respect to Claim 1, Peterson discloses a bioreactor device that includes a growth chamber (10, 50, 100, 150) and means for applying hydrostatic (18, 30) fluid pressure to a cell-seeded scaffold (20) held within the growth chamber; that structures (18, 30) are capable of hydrostatic fluid pressure to cell-seeded scaffolds in the manner recited in the wherein clause of the claim; that the claim does not include positively recited structural elements such as a control device for the means or devices applying the pressure or load to the cell-seeded scaffolds; that with respect to Claims 2-5

the growth chamber is capable of holding any type of scaffold material; that with respect to Claim 6, pump (30) can provide pulsatile flow; that with respect to Claims 22-25, in the absence of further positively recited structure, the device would be capable of providing the claimed tissue; and that with respect to Claims 26 and 27, the device supports the scaffolds within the growth chambers with holding means so as to produce a tissue of a desired shape of a body part can be replace and/or repaired.

Claims 7-12 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Peterson. The Examiner maintains that while Peterson discloses the use of cyclic hydrostatic pressurization loading of the construct held within the growth chamber, the reference is silent as to the specifics of the cyclic treatments in terms of pressures, frequency and/or length of time; that Peterson discloses that the object of the treatment system is to expose the tissue constructs to loading that resembles the physiological conditions typically encountered by the tissue being replaced and/or repaired; and that in view of this teaching, it would have been obvious to one of ordinary skill in the art at the time the invention was made to determine the typical conditions that the desired tissue would be exposed to and operate the device to mimic those physiological conditions in terms of loading, frequency and length of time.

Claims 1-29, 31- 44 and 59-61 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Fofonoff et al., U.S. Patent No. 5,882,929 ("Fofonoff "), in view of Peterson. The Examiner maintains that with respect to Claim 1, Fofonoff discloses a bioreactor device that includes growth chamber (44) and means for applying deformational loading (72, 14) to a cell-seeded scaffold (26, 30) held within the growth chamber; that Claims 1 and 29, as well as Claims 6, 20, 36, 37, and 38, differ because they require that the device and method of use includes a means and step for hydrostatic loading of the scaffold material; that Peterson disloses that it is known in the art to provide a bioreactor device with both a means for deformational loading and hydrostatic

loading of tissue constructs within the bioreactor chamber, specficially that Peterson discloses a bioreactor device that includes a growth chamber (10, 50, 100, 150) and means for applying hydrostatic (18, 30) and deformational loading (12, 54, 56, 154) to a cell-seeded scaffold (20) held within the growth chamber; that in view of this teaching, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the system of Fofonoff to include hydrostatic loading as suggested by Peterson for the known and expected result of providing an additional means recognized in the art for ensuring that the cultured cells are exposed to conditions that mimic physiological conditions and for improving the contact of the culture medium within in pores of the porous scaffold material during the culture process; that the claim does not include any positively recited structural elements such as a control device for the means or devices applying the pressure or load to the cell-seeded scaffolds; that with respect to Claims 2-5, the growth chamber is capable of holding any type of scaffold material; that Claim 1 does not positively recite the scaffold as part of the claimed device; that with respect to Claims 7-12 and 39-44, while Peterson discloses the use of cyclic hydrostatic pressurization and/or loading of the construct held within the growth chamber, the reference is silent as to the specifics of the cyclic treatments in terms of pressures, deformation, frequency and/or length of time; that Peterson discloses that the object the treatment system is to expose the tissue constructs to loading that resembles the physiological conditions typically encountered by the tissue being replace and/or repaired; that in view of this teaching, it would have been obvious to one of ordinary skill in the art at the time the invention was made to determine the typical conditions that the desired tissue would be exposed to and operate the device to mimic those physiological conditions in terms of loading, frequency and length of time; that with respect to Claims 13 the device is also capable of applying an intermittent cyclic strain-controlled deformational loading; and that with respect to Claims 14-19, while Fofonoff discloses the use of cyclic loading of the construct held within the growth chamber, the reference is

silent as to the specifics of the cyclic treatments in terms of pressures, deformation, frequency and/or length of time.

The Examiner also maintains that Fofonoff discloses that the object of the treatment system is to expose the tissue constructs to loading that resembles the physiological conditions typically encountered by the tissue being replaced and/or repaired; that in view of this teaching, it would have been obvious to one of ordinary skill in the art at the time the invention was made to determine the typical conditions that the desired tissue would be exposed to and operate the device to mimic those physiological conditions in terms of loading, frequency and length of time; that the end result is that the structure of the loading device of Fofonoff is capable of providing strain-controlled loading recited in the device claims; that with respect to Claim 21, Peterson discloses that it is known in the art to modify the loads over time in response to changes in the density of the construct; that, as a result, it would have been obvious to one of ordinary skill in the art to modify loads of the modified primary reference over time for the known and expected result of responding to changes in the density of the tissue construct during the culture process; that with respect to Claims 22-25, in the absence of further positively recited structure, the device would be capable of providing the claimed tissue; that with respect to Claims 26-28 and 58-60, the device supports the scaffolds within the growth chambers with holding means so as to produce a tissue of a desired shape of a body part to be replaced and/or repaired; that with respect to Claims 29 and 61, the bioreactor contains scaffold material is impregnated with chondrocyte cells; that with respect to Claims 31, 32, and 34, the reference discloses the use of biocompatible and bioabsorbable materials; that with respect to Claim 35, the resulting constructs mature into replacement cartialge tissue; that with respect to Claim 33, while Fofonoff prefers to use bioabsorable material, the use of synthetic biocompatible material is known; that, as a result, it would have been obvious to one of ordinary skill in the art to use synthetic material for the scaffold for the known and expected result of providing an alternative means recognized

in the art to achieve the same result, supporting the cultured tissue for implantation; and that with respect to Claims 54-57, while the reference is silent as to the specific cartilage produced, in the absence of a showing of criticality and/or unexpected results, it would have been obvious to one of ordinary skill in the art at the time the invention was made to determine the specific type of cartilage produced based merely on the intend use of the cartilage in terms of the location in the body it is intended to be implanted.

Applicants respectfully traverse the above rejections.

Applicants' invention is divided to a bioreactor for generation of load-bearing cartilaginous or fibrocartilaginous tissue by applying deformational loading to scaffolds seeded with chondrocytes and/or other cells. Scaffolds may be shaped to reproduce the geometry of all or part of a load-bearing articular surface or defect as acquired from a database or patient-specific geometry data. A scaffold is optionally attached to a substrate which promotes integration of this tissue construct with the underlying bone of the patient joint. In the bioreactor ambient hydrostatic pressure and scaffold deformational loading can be prescribed with any desired waveform, using magnitudes which prevail in diarthordial joints. The loading platen, permeable or impermeable, may confrom to all or part of the scaffold surfaces. This bioreactor maintains the sterility necessary for the production of bioengineered tissue constructs and provides simultaneous hydrostatic pressure and tissue deformation in a physiologic range, utilizes loading platens which can conform to a designated shape of the tissue construct, and provides for an attachment to promote integration with the underlying bone. Control of matrix strain rather than stress or load is specifically chosen to protect cells from being subjected to levels of deformation that may be detrimental to cell viability and tissue growth during applied loading.

According to Applicants' invention, the deformational loading of cell-seeded scaffolds optionally in a physiologic combination with intermittent hydrostatic pressure results in optimal generation of tissue with functional properties and biochemical composition similar to articular cartilage. A bioreactor useful according to the invention comprises a growth chamber for housing cultured cells, a cell-seeded three-dimensional scaffold, optionally integrated with a substrate that promotes bony ingrowth for attachment to underlying bone, means for applying hydrostatic pressure, and means for applying deformational loading.

The scaffold in the bioreactor supports the growth of a three-dimensional cell culture. The scaffold can be bioresorable, and preferably the substrate is conducive to bony ingrowth.

An aspect of the invention also provides a method for producing functional cartilaginous tissue from a cell-seeded scaffold or a cell-seeded scaffold integrated with an osteoconductive and/or osteoinductive substrate. The method comprises the steps of (a) inoculating chondrocytes or chondroprogenitors into a scaffold or a scaffold integrated with an osteoinductive substrate; (b) placing cell-seeded scaffold or cell-seeded scaffold integrated with an osteoinductive substrate into a bioreactor; (c) filling said bioreactor with liquid growth medium; (d) applying hydrostatic pressurization and/or cyclical deformational loading to the cell-seeded scaffold or cell-seeded scaffold integrated with an osteo-inductive substrate; and (e) culturing said stressed cell-seeded scaffold or cell-seeded scaffold integrated with an osteoinductive substrate for a time sufficient to produce functional cartilaginous tissue.

The physiologically loaded cell-seeded scaffold grown according to this method displays enhanced maintenance of the chondrocyte phenotype. In addition, the cells produce a cartilage-like extracellular matrix.

The Peterson patent is directed to an apparatus and method for the growth of tissue for implantation, primary replacement tendon and ligament tissue constructs. Certain embodiments relate to cartilaginous tissue, where the flow characteristics of media around such tissue is controlled. However, as seen in Figs. 5A to 7C of Peterson, the treatment of cartilaginous tissue according to Peterson is far different from what is disclosed and claimed by Applicants.

Fofonoff discloses an apparatus and method for manufacturing a biopolymer tissue construct. The apparatus comprise two opposing tissue surfaces, separated by a gap that is varied (and decreases only to the point that these two opposing tissue surfaces are just in contact – 0 mm gap). The surfaces can be moved in parallel manner to cause shearing forces, but there is no controlled deformation.

While each of Peterson and Fofonoff each have a broad disclosure relating to tissue growth, neither discloses nor suggests the unique bioreactor or method for the growth of cartilaginous tissue that is described and claimed herein. For example, the growth of tendon or ligament tissue constucts in Petereson has requirements and parameters that are different from the conditions necessary to develop cartilaginous tissue according to the invention.

One of ordinary skill in the art would appreciate that the device described by Peterson is "load or force control". See, "Moreover, the ideal load to be applied necessarily depends from the ..." (Column 7, line 5). Similarly, Fofonoff describes a "load or force control" device; see, "Those of ordinary skill will readily be able to determine in light of the present teachings the type and magnitude of the forces that the tissue is subjected to..." (Column 10, line 44).

A critical distinction needs to be made between the "load or force" control adopted by the devices described by Fofonoff and Peterson and the "deformation" control system prescribed in the present invention. The applied construct stress is proportional to the product of the modulus (e.g., stiffness) of the construct and the strain (applied change in construct dimension normalized by the original undeformed construct dimension in the direction of loading). Under load control, a force is applied (such as with a platen) until such time the prescribed loading level is reached. The platen will move forward until a force arising from the resistance of the construct gives rise to the set load magnitude. For a soft construct (e.g., early in culture), this may only be achieved when the construct is crushed and the top platen becomes in contact with the lower platen on which the construct sits - crushing the specimen. From this scenario, a growing tissue that is significantly softer than native cartilage will undergo non-physiologic deformation during loading that may be detrimental to tissue growth. Alternatively, a non-physiologic load (contrary to that prescribed by Peterson) would have to be applied to preserve the tissue construct (from being overloaded and crushed) when using a "load or force" control system. Neither patent suggests or teaches this concept.

The unique results of the invention have been reported. See, for example, C.T. Hung, R.L. Mauck, C.-C.B. Wang, E.G. Lima, and G.A. Ateshian, A paradigm for functional tissue engineering of articular cartilage via applied physiologic deformational loading, *Ann. Biomed. Eng.* 32(1) (2004), where it is indicated that 3 cycles (1 hour loading followed by 1 hour of rest) at 10% deformation/ 1 Hz per day for 3 days increased aggrecan gene expression 3-fold over free-swelling controls ($p<0.0345$).

With respect to long-term development of construct properties, it was reported by Applicants' laboratory (see, R.L. Mauck, C.-C.B. Wang, E.S. Oswald, G.A. Ateshian, and C.T. Hung, The role of cell seeding density and nutrient supply for articular cartilage tissue engineering with deformational loading, *Osteoarthritis Cartilage* 11(12) (2003),

879-890) that applied deformational loading (10% deformation, 1 Hz, 3 hours/day, 5 days/week) to chondrocyte-seeded agarose constructs seeded at 60 million cells/ml and cultured with high serum concentration (20% FBS) results in a >2-fold increase in material properties relative to free-swelling controls. After two months of culture, dynamically loaded constructs achieved a Young's modulus of ~185 kPa and a dynamic modulus (at 1 Hz) of ~1.6 MPa, with a frequency dependent response similar to that of the native tissue.. These values represent ~3/4 and ~1/4 the values measured for the native tissue, respectively. These results represent the best that have been achieved using mechanical loading.

Representatives from Advanced Tissue Sciences, Inc. (assignee of the Peterson patent) chose to use ± 5% dynamic strain oscillations at 0.001 Hz frequency to stimulate matrix metabolism in tissue engineered cartilage. In their publication, T. Davisson, S. Kunig, A. Chen, R.L. Sah, and A. Ratcliffe, The effects of perfusion and compression on modulation of tissue engineered cartilage, *Trans. Orthop. Res. Soc.* 27 (2002), 488. Followed by T. Davisson, S. Kunig, A. Chen, R. Sah, and A. Ratcliffe, Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage, *J. Orthop. Res.* 20 (2002), 842-848, it was reported that polyglycolic acid (PGA) constructs seeded with 20 million cells for two weeks under free-swelling conditions and then subjected to 9 of deformational loading (confined by a radial ring and loaded between two porous platens in a bioreactor with applied perfusion at (10 μm/sec) and compression with a ± 5% dynamic strain oscillations at 0.001 Hz in a sawtooth pattern) exhibited no significant change in compressive stiffness relative to free-swelling controls. Together, these results demonstrate that the frequency, duration, and magnitude range of deformational loading described and claimed herein is not obvious and is not taught by Fofonoff or Peterson (i.e., references are silent as to the specifics of the cyclic treatments).

Further support that the loading protocol prescribed in the current invention is not obvious can be found in Hunter, S.M. Imler, P. Malaviya, R.M. Nerem, and M.E. Levenston, Mechanical compression alters gene expression and extracellular matrix synthesis by chondrocytes cultured in collagen I gels, *Biomaterials* 23 (2002), 1249-1259, chondrocytes cultured in type I collagen gels and subjected to oscillatory compression (±4%, 1 Hz frequency) for 24 hours exhibited no changes to aggrecan mRNA levels (static loading did).

In sum, the subject matter of the claims herein, especially as amended above, is not suggested or disclosed by the Peterson or Fofonoff patents. Accordingly, the rejections under §102(e) or 103(a) should be withdrawn.

Should the claims herein be allowable but for minor matters that could be the subject of either a supplemental response or an Examiner's Amendment, Applicants would appreciate the Examiner's contacting Applicants' undersigned attorney of record.

Reconsideration and allowance of all the claims herein are respectfully requested.

Respectfully submitted,

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